DEPENDENCE OF THE ANTIPROLIFERATIVE ACTION OF INTERFERON ON ITS DEGREE OF PURIFICATION

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Since the discovery in 1962 that interferon (IFN) preparations can inhibit cell proliferation [13] and synthesis of cellular macromolecules [6] it has been debated whether the effects of this antiproliferative (AP) action are among the properties of IFN itself or whether they are connected with impurities present in IFN preparations [2, 14]. Although it is too early to regard the discussion of this problem as closed, data in recent years indicate that both the antiviral (AV) and the AP effects are characteristic of the IFN molecules themselves. The weightiest argument in support of this view, in the present writers' opinion, is the fact that bacterial (cloned) IFN possess AP activity [1]: Impurities present in it can hardly be identical with those appearing during IFN synthesis in induced eukaryotic cells.

Correlation between the AV and AP activity of IFN is not constant and depends both on the type or subtype of IFN [7, 8] and on the properties of the cellular test systems on which the preparations are investigated [9]. Workers in the writers' Institute have shown that during purification and concentration of human leukocytic IFN (HuLeIFN/NDV) there is a disproportionately large loss of AP activity compared with AV activity; material isolated from IFN and possessing AP activity, moreover, is a low-molecular-weight product [4].

One way of analyzing the causes of variability of the AV/AP ratio in IFN preparations is by experiments on cell cultures differing in sensitivity to IFN. It has been shown on clones of L-1210 mouse cells, some of which remained sensitive to IFN, whereas others lost their sensitivity, that clones resistant to the AV action of IFN are also insensitive to its AP action [11]; an increase in the concentration of mouse IFN, moreover, leads to strengthening of the AP effect in sensitive clones with no effect on proliferation of cells insensitive to IFN [14].

In this investigation the AP action of IFN was studied on cultures of human cells.

EXPERIMENTAL METHOD

Experiments were carried out on human cells of line J-96, sensitive to IFN, and subline J-41, resistant to IFN, obtained from it [5]. The cultures were seeded in Leighton's tubes with coverslips at the rate of 120,000 cells to 1 ml medium 199 with 10% bovine serum. After 24 h of culture HuLeIFN/NDV was added in the form either of the native preparation (specific activity 10^3 IU/mg protein) or concentrated and partially purified IFN (specific activity 10^4 IU/mg protein), or an injection preparation of IFN (specific activity 3×10^6 IU/mg protein). Methods of obtaining and purifying the preparation were described previously [12]; the experiments were so arranged that the final AV activity of all three IFN preparations in the medium was the same, namely 0.001-0.003 IU/cell by titration against 100 TCD_{50} of murine encephalomyocarditis virus. AP activity of the preparations was determined from the mitotic index (MI) and [^{3}H]thymidine incorporation. Details of the method were described previously [3].

EXPERIMENTAL RESULTS

The data in Table 1 show that treatment of J-96 cells sensitive to IFN with each of the three IFN preparations led to marked inhibition of [^{3}H]thymidine incorporation into DNA of

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TABLE 1. Inhibition of [^{3}H]Thymidine Incorporation into Cell Cultures 24 h after Treatment with HuLeIFN/NDV Preparations of Different Degrees of Purity (dose of IFN 0.001-0.003 IU/cell; in %)

IFN preparation	J-96 cells (sensitive to IFN)	J-41 cells (insensitive to IFN)
Native	70,9	60,7
Concentrated	56,6	8,6
Injection	14,4	2,9

Legend. Here and in Table 2 mean results of two or three experiments are given; each experiment was accompanied by its own control. Inhibition expressed in cpm and calculated by the formula $100 - \frac{\text{experiment} \times 100\%}{\text{control}}$.

TABLE 2. Inhibition of Mitotic Activity of Cell Cultures 24 h after Treatment with HuLeIFN/NDV Preparations of Different Degrees of Purity (MI; in $^0/_{00}$)

IFN preparation	J-96 cells (sensitive to IFN)	J-41 cells (insensitive to IFN)
Native	45,3	79,8
Concentrated	47,5	20,0

the cells, but the higher the degree of purification of the IFN preparation, the weaker its effect on synthesis of cellular DNA.

In J-41 cells insensitive to the AV action of IFN, native IFN also caused marked inhibition of incorporation of the precursor, but DNA synthesis was reduced less than by the action of the same preparation on cells sensitive to IFN. Treatment of J-41 cells with concentrated IFN reduced incorporation of [^{3}H]thymidine, but not statistically significantly, whereas after the action of the injection preparation, incorporation was preserved by 97.1%, within the limits of error of the method.

It is stated in the literature that the reduction in [^{3}H]thymidine incorporation in IFN treated cells does not always reflect disturbance of synthesis of cellular DNA: In some types of cells it may be the result of inhibition of membrane transport of exogenous thymidine into the cytoplasm or interruption of several pathways of nucleotide metabolism simultaneously [10]. It was therefore considered necessary to make a parallel study of the reduction in mitotic activity of cultures as a direct indicator of the level of their proliferation. Since the decrease in incorporation of ^{3}H-thymidine in cultures insensitive to IFN after treatment with concentrated IFN was no longer significant, it was decided to compare MI only in cultures treated with native and concentrated IFN preparations (Table 2). The experiments showed that J-96 cells, sensitive to IFN, respond to treatment by both IFN preparations by a significant fall in mitotic activity, whereas in subline J-41, insensitive to IFN, MI fell sharply ($P < 0,001$) on treatment with native IFN, but did not fall significantly ($P \approx 0,05$) after treatment with the concentrated preparation. Consequently, the results indicate real changes in proliferation of the cultured cells.

The results show that concentrated and purified IFN preparations possess AP activity only in cultures of cells sensitive to IFN, and have little or no action on proliferation of insensitive cells. Conversely the native, i.e., unpurified preparation inhibits proliferation of cells both sensitive and insensitive to the AV action of IFN. It undoubtedly follows from

this that the native IFN preparation contains impurities which have AP activity but not AV activity.

According to the definition of the International Committee on IFN Nomenclature, proteins possessing nonspecific antiviral activity at least in cells of the species of animals from which they were obtained, and acting on cell metabolism, including RNA and protein synthesis, are classed as IFN [15]. Hence it follows that any substance which does not possess AV activity, including impurities responsible for much of the AP activity of native preparations, cannot be classed as IFN.

On maximal purification the specific activity of IFN can reach 10^9 IU/mg protein [14], whereas the specific activity of IFN preparations used clinically usually does not exceed 10^6-10^7 IU/mg protein, i.e., these preparations contain only 0.1-1% of true IFN. Consequently, there is reason to suppose that the AP activity of IFN preparations is associated to some degree not with the IFN itself, but with its impurities. In other words, the ability of IFN molecules to inhibit cell proliferation (our findings in no way exclude this ability) constitutes only part of the AP activity exhibited by IFN preparations as a whole. This view is in good agreement both with data given above showing a decrease in AP activity on purification of IFN preparations and also with the fact that the concentrated IFN preparation preserves its very weak AP action on J-41 cells insensitive to IFN. In the writers' view it is therefore worthwhile to isolate and study the properties not only of IFN itself, but also of biologically active impurities contained in IFN preparations.

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